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Simultaneous speciation analysis of Sb(III), Sb(V) and $(CH_3)_3SbCl_2$ by high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry detection (HPLC-HG-AFS): Application to antimony speciation in sea water

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Abstract

This paper presents an improvement for the simultaneous separation of Sb(V), Sb(III) and $(CH_3)_3SbCl_2$ species by high performance liquid chromatography (HPLC) and its detection by hydride generation-atomic fluorescence spectrometry (HG-AFS). The separation was performed on an anion exchange column PRP-X100 using a gradient elution program between EDTA/KHP (potasium hydrogen phtalate) as first mobile phase and phosphate solutions solution as the second one. The chromatographic separation and the HG-AFS parameters were optimized by experimental design. The best results were obtained by using an elution program with 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP solution at pH 4.5, during 1.15 min, then change to 50 mmol l^{-1} $(NH_4)_2HPO_4$ solution at pH 8.3, switching back after 4.0 min to the first mobile phase, until 5 min, with a constant flow rate of 1.5 ml min^{-1}. Retention time of Sb(V), Sb(III) and trimethylantimony species were 1.22, 2.31 and 3.45 min and the detection limits were 0.13; 0.07 and 0.13 μg l^{-1}, respectively. Studies on the stability of this antimony species in sea water samples on the function of the elapsed time of storage in refrigerator at 4 °C was performed employing the optimized method. Results revealed that Sb(III) is easily oxidized within some hours to Sb(V) in sea water stored at 4 °C. However, when the sea water was immediately mixed with EDTA no oxidation of Sb(III) was observed up to 1 week of storage. The proposed methodology was then applied to the antimony speciation in sea water samples.

Keywords: Antimony; Speciation; HPLC; Hydride generation-atomic fluorescence spectrometry; Stability; Sea water

1. Introduction

Antimony is a non-essential element in plants, animals and humans [1]. Occupational exposure to antimony compounds is known to cause adverse health effects in humans and animals [2,3].

In environmental samples beside the two inorganic antimony species, Sb(III) and Sb(V), methylated forms have been detected [4–8]. In sea water, methylantimony species represent about 10% of the total dissolved antimony [9–11].

Speciation of antimony is of great importance owing to the large differences regarding their toxic properties. Elemental antimony is more toxic than its salts, and generally, trivalent antimony compounds exert a toxicity that is 10 times higher than the pentavalent antimony species [1,12].

Most of the analytical techniques for the separation and detection of antimony species are based on the line coupling of high-performance liquid chromatography (HPLC)

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to element-specific detectors, such as a hydride generation-atomic absorption spectrometer (HG-AAS) [13–15], hydride generation-atomic fluorescence spectrometer (HG-AFS) [16–18], inductively coupled plasma-optical emission spectrometer (ICP-OES) [5] or to inductively coupled plasma-mass spectrometer (ICP-MS) [5,7,19–27]. Methodologies for speciation analysis of antimony have been reviewed by Smichowski et al. [28] and Nash et al. [29]. More recently, Krachler et al. [30] presented a review on the antimony speciation, focusing on hyphenated instrumental techniques, as well as the problems encountered. Antimony speciation methods based on anion exchange chromatography have led to the successful separation of aqueous Sb(III) and Sb(V) or Sb(V) and $(CH_3)_3SbCl_2$. In general, the elution of Sb(V) is easily achieved under different chromatographic conditions; while for Sb(III), long retention time, irreversible retention and severe peak tailing have been encountered. These problems have been partially solved by using complexing mobile phases, such as ethylenediamine-tetraacetic acid (EDTA) [15,21,26,27], EDTA mixed with potassium hydrogen phthalate (KHP) [21,22,27], tartrate [15,27,31] and citrate [22] buffer solutions. Furthermore, the elution of $(CH_3)_3SbCl_2$ is only achieved by using basic mobile phases, such as carbonate buffer, phosphate buffer, potassium hydroxide or tetramethylammonium hydroxide [8,19,27]. So far, only few analytical methodologies are reported regarding the simultaneous separation and on line determination of the two inorganic antimony species Sb(III), Sb(V) and the only trimethylated antimony standard $(CH_3)_3$ $SbCl_2$ or $(CH_3)_3$ $SbBr_2$ currently available to the scientific community [7,8,15–18,20]. Zheng et al. [8] showed that these three antimony species could be separated on an Asahipak HG-520 SEC column using 50 mmol l^{-1} of Tris buffer solution, pH 7.4. Sayago et al. [16] described firstly the separation of Sb(V) and Sb(III) and then, the optimization of the separation of Sb(V), Sb(III) and $(CH_3)_3SbBr_2$ on an anion exchange PRP-X100 column using HG-AFS as detection technique [17]. The separation was achieved by using a concentration gradient elution between 20 mmol l^{-1} potassium hydroxide, pH 11, and ammonium tartrate, with a concentration as high as 200 mmol l^{-1}, at pH 5 [17]. Under these experimental conditions, some chromatographic problems still remain, such as elution of $(CH_3)_3$ $SbBr_2$ in the void volume, insufficient peak resolution and severe peak tailing for Sb(III) [17]. Recently, Miravet et al. described a method for the speciation analysis of Sb(III), Sb(V) and $(CH_3)_3SbCl_2$ based on the same separation and detection techniques (HPLC-HG-AFS) [20]. The maximum efficiency and resolution were obtained using, as the mobile phase, a gradient elution between 250 mmol l^{-1}diamonium tartrate, pH 5.5 and 20 mmol l^{-1} KOH, pH 12. The analysis took about 7 min. The methodology was applied to antimony speciation in fresh waters [18]. From results presented in this paper, a non quantitative separation between trimethylantimony and Sb(III) species can be deduced, in spite of the high diamonium tartrate concentration and pH of the mobile phases employed.

This paper presents, based on a similar chromatographic approach, an improvement of the simultaneous separation of Sb(III), Sb(V) and $(CH_3)_3SbCl_2$ on an exchange PRP-X100 column using mobile phases of lower concentration and pH and subsequent post column sensitive detection by hydride generation atomic fluorescence spectrometry (HPLC-HG-AFS). Atomic fluorescence spectrometry (AFS) can be a good alternative to inductively coupled mass spectrometry (ICP-MS) detector, with the advantage of a lower cost of investment and handling. The separation of the antimony species by using a gradient elution between EDTA + KHP (potassium hydrogen phthalate) and different phosphate solutions was investigated. The separation conditions were optimized by experimental design, in order to achieve good efficiency and resolution within a short analysis time. The optimized methodology was then applied to study the stability of these antimony species in sea water samples, matrix where the antimony speciation has been few studied, probably due to the high chloride concentration which leads to detection interferences, especially using ICP-MS detection. The methodology was applied to antimony speciation in sea water samples collected from Valparaíso bay.

2. Experimental

2.1. Chemicals and reagents

For the preparation of all solutions, high purity water (18 MΩ) from a Nanopure system (Barnstead, Dubuque, IA, USA) or a Milli-Q (Millipore, Bedford, MA, USA) system was used. Chemicals were of analytical-reagent grade or higher purity. Glass and plastic wares were cleaned by soaking for 1 day in 10% (v/v) nitric acid (analytical grade) and were rinsed several times with high purity water before use.

Antimony (III) standard was obtained as potassium antimonyl tartrate $K(SbO)C_4H_4O_6H_2O$ (Aldrich, 99.95% purity); a standard solution of 100 mg l^{-1} Sb(III) was prepared daily by dissolving this compound in water. A stock solution of Sb(V) was prepared by dissolving solid potassium hexahydroxo-antimonate $KSb(OH)_6$ (Aldrich, 99.95% purity) in water. Trimethylantimony dichloride $(CH_3)_3SbCl_2$ was purchased from Aldrich (96% purity). Stock standard solutions were prepared in water to give 100 mg Sb l^{-1}. All standard solutions were stored in polyethylene bottles at 4 °C. Working antimony solutions were prepared daily by an appropriate dilution in the mobile phase (20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP).

The mobile phases were freshly prepared as follows: the mixture of 20 mmol l^{-1} EDTA + 2 mmol l^{-1} potassium hydrogen phthalate (KHP, Merck) was prepared by dissolving di-sodium dihydrogen ethylene diamine tetraacetate salt dihydrate (Na_2 EDTA·$2H_2O$, Merck) and potassium hydrogen phthalate in high purity water. Different mixtures of diammonium hydrogen phosphate and ammonium di-hydrogen phosphate ($(NH_4)_2HPO_4$ + $(NH_4)H_2PO_4$) were

prepared by dissolving the respective solid salts (Merck). $(NH_4)_2HPO_4$ solutions of different concentrations were also employed as mobile phases. The mobile phases were filtered on 0.45 μm membrane filters, (Millipore, type HA) and degassed by sonication before use.

For the hydride generation system coupled to HG-AFS, the carrier solution (3 mol l^{-1} HCl) was prepared from concentrated hydrochloric acid (Merck). $NaBH_4$ solutions (3%, m/v) were prepared daily by dissolving appropriate amounts of powdered $NaBH_4$ (analytical-reagent grade, Merck) in 0.4% (m/v) NaOH solution (Merck).

2.2. Instrumental and conditions

The chromatographic separation of antimony species was performed with a Hewlett Packard HPLC system 1050 model equipped with quaternary pumps, degassers, autosampler and injector with a 100 μl loop. To separate Sb(III), Sb(V) and $(CH_3)_3SbCl_2$ a short Hamilton PRP-X100 column (100 mm × 4.1 mm) was used.

Hydride generation of volatile stibines was carried out by the on line addition of 3 mol l^{-1} HCl and 3% (m/v) $NaBH_4$ solutions at the outlet of the column by means of the two peristaltic pumps of the HG-AFS system (PSA Analytical Excalibur Millenium system). An argon flow of 300 ml min^{-1} was fixed to carry the volatile stibines generated into the gas liquid-separator to the detector. Before detection, a secondary argon flow (40–80 ml min^{-1}) and a supplementary hydrogen flow were injected (40–80 ml min^{-1}) to maintain a stable argon/hydrogen diffusion flame. The gas flow was dried through a hygroscopic membrane drying tube (Perma Pure product, dryer model MD-110-12 FP). A boosted discharge antimony hollow cathode lamp (Sb BDHCL Super lamp, Photron, Victoria, Australia) was used as the radiation source of the atomic fluorescence detector. For the data acquisition an interface Hercules was installed, piloted by a computer equipped with Borwin software. As the Hercules interface needed an analogical signal and the output of the AFS detector is a digital signal, a digital–analogical converter 0–1 V (PSA) was placed between both systems. The HPLC system and departure of the AF signal was synchronized by the Hercules interface. This configuration makes it possible to program the injection as well as the switch of mobile phases. The ICP-MS instrument used was an Agilent 7500 Series. The optimal conditions employed are summarized in Table 1.

Table 1
Summary of optimal conditions for HPLC coupled to HG-AFS and HG-ICP-MS detection system

HPLC (Hewlett Packard HPLC system, 1050 model)	
Column	Hamilton PRP-X100 (100 × 4.6 mm id, particle size 5 μm)
Mobile phases	First mobile phases: 20 mmol l^{-1} EDTA + 2 mmol l^{-1} potassium hydrogen phthalate (KHP), second mobile phase 50 mmol l^{-1} $(NH_4)_2HPO_4$ solution
Flow rate (ml min^{-1})	1.5
Injection volume (μl)	100
HG-AFS (PS Analytical LTDA, Millennium Excalibur system)	
Sb BDHCL	
Primary current (mA)	18
Boosted (mA)	15
HCl	3 mol l^{-1} (0.40 ml min^{-1})
$NaBH_4$	3%, m/v (in 0.4% NaOH) (0.25 ml min^{-1})
Argon flow (primary) (ml min^{-1})	300
Secondary (ml min^{-1})	40
Hydrogen (auxiliary) (ml min^{-1})	40
ICP-MS (Agilent 7500 Series)	
Forward rf power (W)	1100
Plasma argon flow (l min^{-1})	15
Auxiliary flow (l min^{-1})	0.9
Flow to gas liquid-separator (l min^{-1})	1.0
Torch gas (l min^{-1})	0.1
Integration time (s $isotope^{-1}$)	0.4
Isotopes monitored	^{121}Sb and ^{123}Sb
Total analysis time (s)	300

3. Results and discussion

3.1. Chromatographic behavior of antimony species (Sb(III), Sb(V) and $(CH_3)_3SbCl_2$ using different mobile phases

As in Lintschinger et al. [27] a complexing mobile phase containing 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP, pH 4.5 was chosen for preliminary experiments. It is shown in Fig. 1A that Sb(V) and Sb(III) present well defined peaks using this mobile phase, with retention times of 1.1 and 1.7 min, respectively. However, the separation between Sb(V)-Sb(III) species is not quantitative (resolution (Rs) <1.2), especially when Sb(V) concentration is higher than Sb(III) (i.e. for [Sb(V)]/[Sb(III)] ratio = 10, Rs = 1.0). Otherwise, trimethylantimony species were retained on the column under the aforementioned chromatographic conditions. Then, based on results reported by Lintschinger et al. [27], the chromatographic behavior of each antimony species was determined using phosphate buffer $(NH_4)_2HPO_4/(NH_4)H_2PO_4$ solutions from 0.5 to 5 mmol l^{-1}, pH 6.9 as the mobile phase.

It is shown in Fig. 1B that Sb(III) is irreversibly retained on the column when 30 mmol l^{-1} $NH_4H_2PO_4$ + 30 mmol l^{-1} $(NH_4)_2HPO_4$ solution at pH 6.9 was used as the mobile phase, while Sb(V) and trimethyl antimony species are eluted with retention times of 1.2 and 1.5 min, respectively. The resolution between these species is not quantitative (Rs = 0.6).

From these preliminary results, it was deduced that the simultaneous separation of the three antimony species would require a gradient elution of the mobile phase. So, a chromatographic gradient was developed, consisting as the first mobile phase 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP, pH 4.5

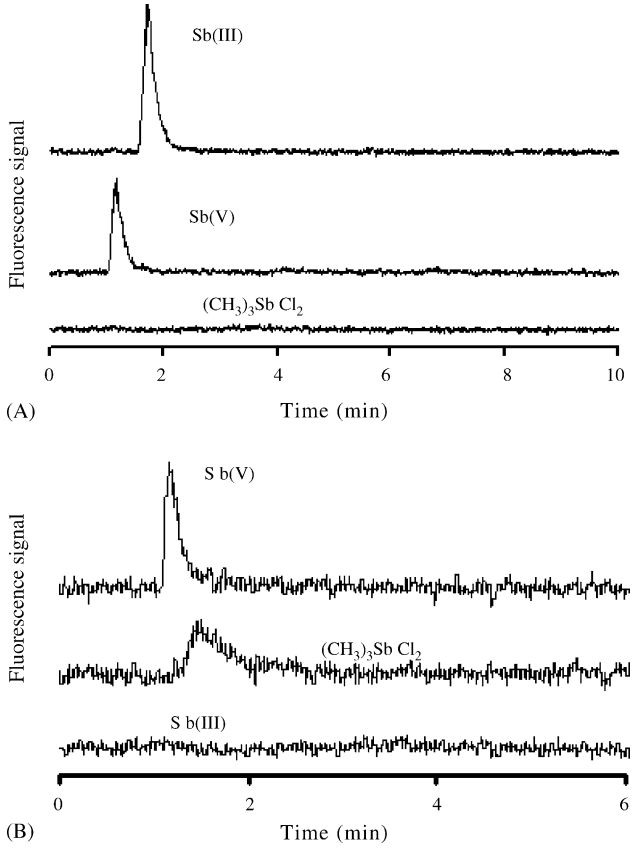

Fig. 1. Chromatogram of each antimony species (5 μg Sb l^{-1}) using PRP-X100 column and as mobile phases: (A) 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP solution, pH 4.5; (B) 30 mmol l^{-1} $NH_4H_2PO_4$ + 30 mmol l^{-1} $(NH_4)_2HPO_4$ buffer solution at pH 8.3 (flow rate 1 ml min^{-1}).

to elute Sb(V) and Sb(III) and as the second mobile phase 30 mmol l^{-1} $NH_4H_2PO_4$ + 30 mmol l^{-1} $(NH_4)_2HPO_4$ buffer solution, at pH 6.9 to elute trimethylantimony. The chromatographic gradient employed is presented in Table 2. It was postulated that by switching the first mobile phase to the phosphate buffer after 1 min, the retention time of Sb(III) would be increased, obtaining a better separation between Sb(III) and Sb(V), and trimethylantimony could then be eluted by this last mobile phase.

The simultaneous separation of the three antimony species was successfully obtained under these chromatographic conditions. The retention times of Sb(V), Sb(III) and trimethylantimony species were 1.26, 2.82 and 7.13 min, respectively. The t_0 value 1.01 min was measured by injecting a synthetic solution of trimethylarsine oxide, a cationic compound that is not retained by the stationary phase and detected by HG-AFS, using an arsenic lamp as the radiation source [32]. The resolution factors for Sb(V)-Sb(III) and Sb(III)-trimethylantimony species were 1.6 and 3.2, respectively. However, the retention time for trimethylantimony species remains high under these conditions, inducing a long analysis time. Then the effect of the total phosphate buffer concentration on the retention time of the trimethylantimony species was investigated within the range of 20–80 mmol l^{-1}, maintaining the $NH_4H_2PO_4/(NH_4)_2HPO_4$ concentration ratio equal to 1, at pH 6.9. However, these changes did not improve the chromatographic behavior of the trimethylantimony species. Thus, the eluting strength and pH of the second mobile phase were then modified; changing the phosphate buffer solution to a $(NH_4)_2HPO_4$ solution, at pH 8.3. It is shown in Fig. 2 that applying the same chromatographic gradient, the retention time of trimethylantimony species decreases when the $(NH_4)_2HPO_4$ concentration increases from 25 to 80 mmol l^{-1}, maintaining the pH solution at 8.3.

Table 2
Chromatographic gradient elution used to separate Sb(V), Sb(III) and trimethylantimony species by HPLC and HG-AFS detection, mobile phase flow rate 1 ml min^{-1}

Time (min)	% 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP, pH 4.5	% 30 mmol l^{-1} $NH_4H_2PO_4$ + 30 mmol l^{-1} $(NH_4)_2HPO_4$, pH 6.9
0.00	100	0
1.00	100	0
1.01	0	100
6.00	0	100
6.01	100	0
10.00	100	0

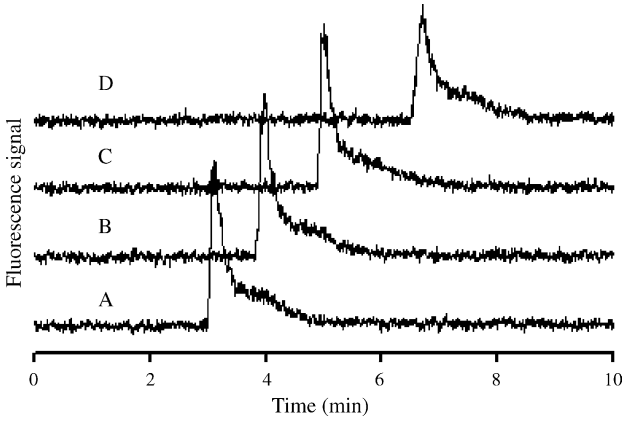

Fig. 2. Chromatograms of trimethylantimony species (10 μg Sb l^{-1}) on the function of $(NH_4)_2HPO_4$ concentration at pH 8.3 (A) 80, (B) 65), (C) 50 and (D) 25 mmol l^{-1} (PRP-X100 column and gradient elution described in Table 2).

It was found that when the separation of the three antimony species was performed changing the second mobile phase to 80 mmol l^{-1} $(NH_4)_2HPO_4$, pH 8.3, the retention times of Sb(III) and trimethylantimony were 2.8 and 3.0 min, respectively, leading to overlapping peaks. In order to obtain quantitative separation between the antimony species and a short analysis time, the elution conditions were optimized.

3.2. Optimization of the chromatographic separation

A first mobile phase containing a concentration of 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP, pH 4.5 was used throughout the optimization procedures. The parameters evaluated in this study included: the concentration of

Table 3
Summary of chromatographic variable ranges and analysis of variance on the resolution of Sb(III)-$(CH_3)_3SbCl_2$ signals; height/width ratio of Sb(V), Sb(III) and trimethylantimony (TMSb) signals and t_R trimethylantimony responses

Codified variables	Symbol	−1	1
Elution time with EDTA-KHP (min)	X1	0.9	1.5
Elution time with $(NH_4)_2HPO_4$ (min)	X2	3.0	4.0
$(NH_4)_2HPO_4$ concentration mmol l^{-1}	X3	30	50
Flow (ml min^{-1})	X4	1.0	1.5

Variables and interaction	Resolution Sb(III)–TMSb	Coefficients (height/width)			Retention time TMSb
		Sb(V)	Sb(III)	TMSb	
X1	**1.33**	**1924**	0.84	−602	**0.41**
X2	**−0.61**	**−1872**	**115.78**	**2509**	**−0.18**
X3	**−0.59**	**−6601**	**42.99**	23	**−0.19**
X4	**0.54**	**9038**	**323.63**	**5901**	**−0.44**
X1X2	**−0.22**	609	0.71	562	−0.03
X1X3	0.06	**−1562**	0.84	−379	0.02
X1X4	0.02	**−2535**	**5.62**	810	−0.02
X2X3	−0.17	**−1665**	**3.95**	**−4016**	−0.02
X2X4	−0.01	**2854**	**13.5**	**2964**	−0.02
X3X4	0.001	**−4448**	**4.94**	**1477**	−0.03
X1X1	−0.08	**−1600**	0.03	**−7648**	0.06
X2X2	−0.02	−1044	**4.24**	**−3597**	0.04
X3X3	**0.52**	1055	**16.99**	**5243**	0.08
X4X4	0.10	**9256**	**10.37**	**2852**	0.10
Constant	3.65	29900	17382	25015	4.06
R-squared	0.98	0.98	0.98	0.97	0.98

$(NH_4)_2HPO_4$ at pH 8.3, the chromatographic gradient elution, and the flow rate of the mobile phases. This study was based on a factorial experimental design at two levels (2^4), across a face-centered experimental design model, including 27 experiences. The experiments were performed in duplicate, in a random manner to avoid any systematic error. The experimental field of each parameter is presented in Table 3. As results showed that the Sb(V)-Sb(III) resolution does not change significantly, for the optimization, only the resolution of Sb(III)-trimethylantimony peaks was considered. The precision and significance of the corresponding fitting of the different responses are shown in Table 3.

Results demonstrated that the four variables, in the studied ranges, had a significant effect on the resolution of Sb(III) and trimethylantimony species (*p*-values <0.05 are shown in bold type in Table 3). Furthermore, the interaction (X1X2) between the elution times with the different mobile phases also had a significant influence on the resolution. The statistical weight of the quadratic interaction of $(NH_4)_2HPO_4$ concentration (X3X3) assures that the model is not linear. Furthermore, by switching the EDTA + KHP eluting solution to a $(NH_4)_2HPO_4$ solution at 1.0 min and maintaining it for 4 min, the Sb(III) and trimethylantimony species separation was quantitative (Rs > 1.5). However, in the resolution responses, the quality of the chromatographic signals was not considered. For this reason, the parameter height/width ratio of chromatographic peaks for each antimony species was included, within the same range of variables. Results presented in Table 3 show that the flow rate of the mobile phase (X4) is the main variable that affects the height/width ratio of the antimony species signals. High flow rate provided a significant response improvement.

An important parameter to be considered in a chromatographic separation is the duration of the analysis. Results evidenced that the elution time with EDTA + KHP solution (X1) and the flow rate of the mobile phase (X4) were the most significant variables on the analysis duration. The retention time of trimethylantimony species decreased at short elution time using EDTA + KHP as well as when the $(NH_4)_2HPO_4$ concentration was increased.

Based on the resolution and the height/width ratio of the chromatographic peaks and the analysis times, a compromise between the different variables was made. The conditions retained were: elution with 20 mmol l^{-1} EDTA + 2 mmol l^{-1} KHP at pH 4.5 solution for 1.15 min, then changing to a 50 mmol l^{-1} $(NH_4)_2HPO_4$ solution at pH 8.3, switching back after 4.0 min to the first mobile phase until 5 min, with a constant flow rate of 1.5 ml min^{-1}. Thus, with the application of this program, the analysis duration is only 5 min.

3.3. Optimization of the hydride generation system

To achieve the best analytical performance for the on line detection of antimony species, the following parameters of the HG-AFS system were optimized by applying the optimal separation conditions. The concentrations of HCl and $NaBH_4$ solutions were fixed at 3 mol l^{-1} and 3% (m/v), respectively, in order to avoid high flow rate of reagents with the risk of high pressure in the thin tubing, and thus disconnection. The fluxes optimized were (ml min^{-1}): HCl (0.4–0.6),

Table 4
Analysis of Variance in the optimization of the signal/background ratio response for the Sb(V), Sb(III) and trimethylantimony (TMSb) peaks in the HG-AFS detection system

Fluxes	Symbol	−1	1
HCl 3 mol l^{-1}	Y1	0.4	0.6
$NaBH_4$ 3% (m/v)	Y2	0.25	0.5
H_2	Y3	40	80
Secondary Ar	Y4	40	80

Variable	Coefficients		
	Sb(V)	Sb(III)	TMSb
Y1	−3.13	−1.84	−2.25
Y2	−3.80	−8.91	−7.06
Y3	−10.40	−16.72	−12.10
Y4	−1.87	−6.78	−3.89
Y1Y2	2.128	−0.62	2.70
Y1Y3	1.82	1.30	1.63
Y1Y4	1.23	1.58	2.40
Y2Y3	2.01	3.63	4.44
Y2Y4	3.81	5.78	3.10
Y3Y4	2.05	7.67	3.47
Y1Y1	−0.33	−2.32	−0.39
Y2Y2	−0.84	−3.72	−1.90
Y3Y3	2.49	8.75	1.22
Y4Y4	−1.77	2.38	1.17
R-squared	0.98	0.98	0.99

$NaBH_4$ solution (0.25–0.50), H_2 supplied to stabilize the flame (40–80) and secondary Ar (gas arriving to separator) (40–80). The criterion for choosing the optimum operational conditions in this study was based on the maximum signal-to-background ratio (S/B) of each antimony species. The optimization was performed using a face-centered full central composite design (with two centers), including 26 experiments, each of them carried out in duplicate and randomly. The studied experimental field of each variable and results are presented in Table 4. The most significant variables for the signal/background ratio of the three antimony species were the flow rate of H_2 and $NaBH_4$. The optimal fluxes were: 0.4 ml min^{-1} HCl, 0.25 ml min^{-1} $NaBH_4$, 40 ml min^{-1} H_2 and 40 ml min^{-1} Ar. Fig. 3 shows the chromatogram obtained with the optimized separation and detection conditions.

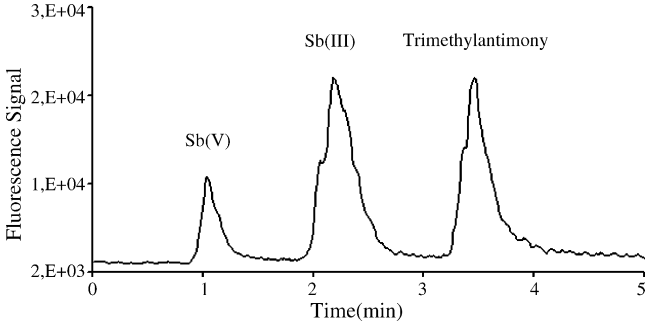

Fig. 3. Chromatogram of the separation of Sb(V), Sb(III) and trimethylantimony species (10 μg Sb l^{-1} each species) using PRPX-100 column and the optimized conditions.

3.4. Analytical characteristics

In the optimal experimental conditions, linear calibration curves between the concentration of each antimony species and the peak area were obtained within 0.5–200 μg l^{-1}, with correlation coefficients better than 0.99. The detection limits calculated according to IUPAC criteria as the concentration corresponding to three times the standard deviation of 10 blank solutions (3 SD of blank signal/slope) for Sb(V), Sb(III) and trimethylantimony species were 0.13, 0.07 and 0.13 μg l^{-1}, respectively, for an injection loop of 100 μl. In spite of the similar signals obtained for Sb(III) and trimethylantimony species, the detection limit for the later was higher, due to the higher background noise originated by the second mobile phase. The precision expressed as the relative standard deviation (RSD) was assessed by analyzing solutions of 5 μg l^{-1} of each standard solution, for a cycle of injections made in triplicate per day, over 5 days. This was near 5% for each antimony compound. The retention times for Sb(V), Sb(III) and trimethylantimony were 1.22 ± 0.01, 2.31 ± 0.08 and 3.45 ± 0.07 min, respectively.

These characteristics were compared with those obtained by using the same chromatographic and hydride generation conditions coupled to an ICP-MS detector. The measurements were made for the isotopes ^{121}Sb and ^{123}Sb. The precision obtained by both methods is similar; (RSD 5–6%); the limits of detection for Sb(V) and Sb(III) obtained by HG-ICP-MS, for both isotopes, were 0.02 and 0.04 μg l^{-1}, respectively. The LODS for trimethylantimony species were 0.13 and 0.16 μg l^{-1} for ^{121}Sb and ^{123}Sb, respectively. These values are comparable to those obtained by using HG-AFS detection, probably because hydride generation is the limiting step controlling the obtained sensitivity. It should be noted that the on line detection of eluted Sb species by ICP-MS, without previous hydride generation was not possible, due to the high salt content of the second mobile phases employed (50 mmol l^{-1} $(NH_4)_2HPO_4$ solution). A similar problem was observed in the antimony speciation in sea water samples.

Compared to the other methods previously reported, the optimized method for antimony speciation, based on the same chromatographic and detection systems, presents the following advantages: use of mobile phases of less aggressiveness (lower concentration and pH), quantitative resolution between the three antimony species and shorter analysis duration (5 min) including the equilibration times.

3.5. Application of the optimized methodology to antimony speciation in sea water

Validation of analytical methods for antimony speciation is difficult owing to the absence of certified reference material for the species of this element. Another severe problem hampering the speciation of antimony compounds is the lack of stability of compounds through the analytical process. Little attention has been paid to sample storage and preparation.

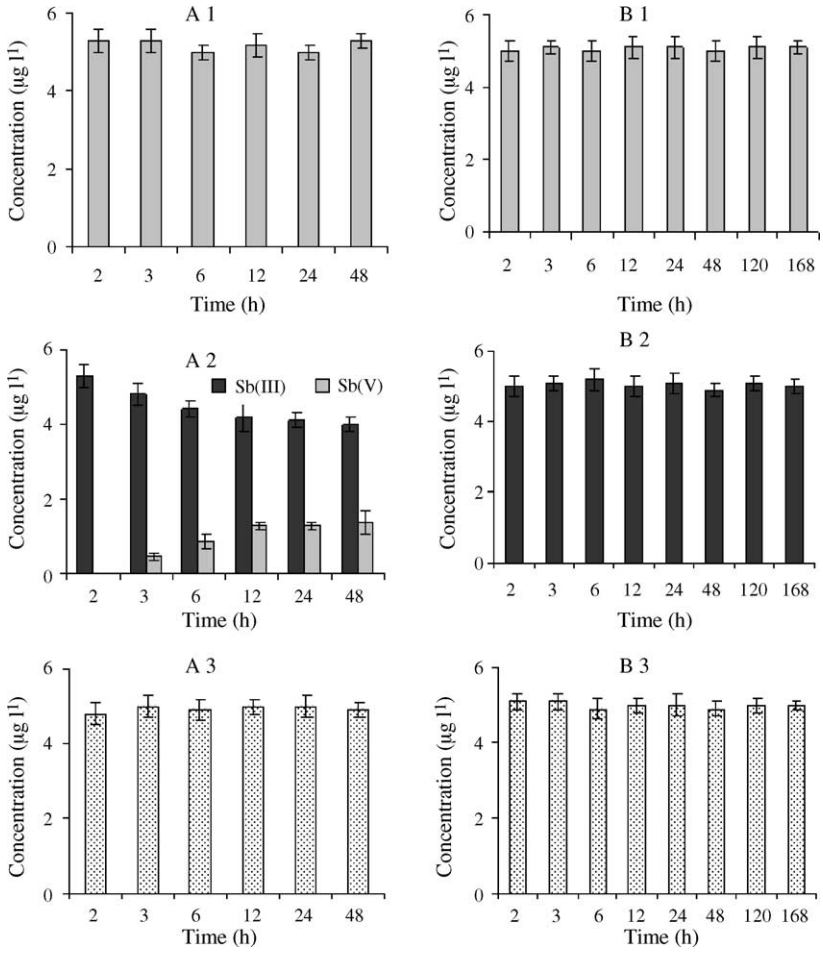

Fig. 4. Concentrations of antimony species determined in sea water samples on the function of elapsed storage times, at 4 °C. (A) Samples without EDTA; (B) samples treated with 20 mmol l^{-1} EDTA spiked with 5 μg l^{-1} of: (1) Sb(V), (2) Sb(III), (3) trimethylantimony species.

For antimony speciation the main difficulty encountered is the oxidation of Sb(III) to Sb(V). However, to date, no detailed studies on the stability of the three antimony species in sea water samples have been published. Therefore, a study on the stability of Sb(V), Sb(III) and trimethylantimony species in sea water samples during the storage was achieved. For this purpose two different sea water samples, collected from different sites (filtered through a 0.45 μm membrane filter) were first analyzed for antimony species using the proposed HPLC-HG-AFS method. Only Sb(V) was detected in one of them (2.2 ± 0.1 μg l^{-1}). In order to investigate the possible species changes during storage, the experiments were carried out in the sea water samples in which no antimony species were detected. The sea water samples were spiked with 5 μg Sb l^{-1} of Sb(III), Sb(V) and trimethylantimony, separately. Samples were analyzed using the standard additions method, to prevent possible matrix effects, after different elapsed times of refrigerated storage at 4 °C. Results demonstrated that 8 and 15% of the spiked Sb(III) was oxidized to Sb(V) within 4 and 48 h, respectively. Since an oxidation of Sb(III) was observed, the speciation analysis of antimony was carried out in a fresh, filtered sea water sample without or with 20 mmol l^{-1} EDTA, spiked with 5 μg Sb l^{-1} of each antimony species, separately. It is shown in Fig. 4A that in sea water samples without EDTA, Sb(III) was oxidized to Sb(V) as a function of storage times. After 6 h, about 20% of the spiked Sb(III) was already oxidized to Sb(V). However, in sea water samples treated after filtration with 20 mmol l^{-1} EDTA, the results (Fig. 4B) clearly demonstrate that Sb(III) is stabilized. EDTA was shown to be an effective reagent for this purpose. No change in the antimony species concentrations could be observed even 1 week after the spiking experiments. This approach involves the probable conversion of Sb(III) into stable Sb(III)-EDTA complexes, in a sea water medium [33].

From an analytical point of view, this result is important when taking into account that the speciation analysis of antimony in sea water samples (collected at sites far away from the laboratory) could be performed up to 1 week after collection, providing that the samples were filtered and mixed with EDTA in situ and stored at 4 °C until analysis.

The optimized methodology was then applied to antimony speciation in sea water samples collected at different

places from the coastal area of Valparaíso bay, including a sampling site at Valparaíso harbour. The total antimony concentrations determined by HG-AFS, in the experimental conditions reported by Chen et al. [34], ranged between 0.03 and 0.55 $\mu g\,l^{-1}$. In samples where the antimony species were detected, the predominant species was Sb(V), excepting in the sample collected at Valparaiso harbour. In this latter, with a total antimony concentration of $0.55 \pm 0.04\ \mu g\,l^{-1}$, the Sb(V) and Sb(III) concentrations determined, using the standard addition method, were 0.20 ± 0.03 and $0.23 \pm 0.04\ \mu g\,l^{-1}$, respectively. In one sample the trimethylantimony species was detected, but its concentration was below the limit of detection. It is important to remark that no change in the retention times of the antimony species was observed when the sea water samples mixed with EDTA were analyzed.

4. Conclusions

The methodology described represents an improvement for the separation of Sb(V), Sb(III) and trimethylantimony species on a PRP-X100 anion exchange column, using a gradient elution made of EDTA-KHP and $(NH_4)_2HPO_4$ and its on-line determination by HG-AFS. The experimental conditions for the separation of antimony species by HPLC and its detection by HG-AFS were optimized by experimental design. The optimized method offers good resolution and detection limits within a short analysis time (5 min including the equilibration time of the column).

Regarding the stability of antimony species in sea water, it can be concluded that Sb(III) is oxidized to Sb(V) during storage at 4 °C. This oxidation is avoided by mixing the filtered sea water samples with an EDTA solution. EDTA was shown to be an effective stabilizing reagent to avoid the oxidation of Sb(III) to Sb(V) in sea water samples during storage, allowing to carry out the speciation analysis up to 1 week after sample collection.

Future investigations will be focused on the application of the optimized methodology to antimony speciation to other environmental matrices.

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